Docket No. 55107 (71526)

APPLICANT: S. Mori et al. SERIAL NO: 09/674,337

FILED: July 26, 2001

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AMENDMENTS TO THE SPECIFICATION

Please amend the specification as shown.

In the specification:

Please delete the paragraph on page 4, lines 11-14, and replace it with the following paragraph:

Fig. 5 shows a comparison with a six partial amino acid sequence determined by nicotianamine synthase originated from barley and similar sequence of graminaceous plants obtained by computer search of the database. Identical amino acid residue is shown in ":"_(SEQ ID NOS 30-37, respectively in order of appearance).

Please delete the paragraph on page 4, lines 15-19, and replace it with the following paragraph:

Fig. 6 shows full length of HvNAS1 cDNA (SEQ ID NO: 2) and amino acid sequence (SEQ ID NO: 1) deduced therefrom. The underlined sequences indicate the identical partial amino acid sequences of fragments in the above Fig. 5. Numbers of the nucleotide sequence are indicated to the right of each row. Amino acid numbers are indicated on the left of each row.

Please delete the paragraph on page 4, lines 20-22, and replace it with the following paragraph:

Fig. 7 shows comparison of the deduced amino acid sequences of the above 7 cDNA obtained from barley (SEQ ID NOS 7, 13, 11, 3, 5, 1, and 9, respectively in order of appearance). Asterisks "*" indicates identical amino acid residues in all sequences used to generate consensus sequences SEQ ID NOS: 23-29.

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At page 26, line 6, please replace the paragraph immediately following the subheading "Example 9 (Preparation of polyclonal antibody)" with the following paragraphs to add and modify the text as shown:

-- Example 9 (Preparation of polyclonal antibody)

Two rats were immunized using the antigen containing about 100 µg of isolated nicotianamine synthase. The antigen was the same sample as that determined the partial amino acid sequence. The complete Freund's adjuvant was used at the first immunization and the incomplete Freund's adjuvant was used since the second immunization. All the constituents of the blood were corrected after the rats were immunized four times, and the obtained serum was preserved at -80°C.

Example 10 (Western blotting analysis)

Total protein was extracted using trichloroacetic acid and acetone (Damerval et al. 1986). The plants were crashed in the liquid nitrogen until powder was obtained, and mixed with acetone containing 0.1% (v/v) 2-mercaptoethanol. The protein was precipitated by allowing to stand at -20°C for 1 hour, and the precipitate was collected by centrifugation at 16,000 X g for 30 minutes. The precipitate was suspended in acetone containing 0.1% (v/v) 2-mercaptoethanol and allowed to stand at -20°C for 1 hour, then collected the precipitate by centrifugation at 16,000 X g for 30 minutes. The precipitate was dried in vacuo, and dissolved in the sample buffer [9.5 M urea, 2% (w/v) Triton X-100 and 5% (v/v) 2-ME], then centrifuged at 16,000 X g for 10 minutes to obtain the supernatant. The proteins contained in the supernatant were separated by SDS-PAGE or the denaturing two-dimensional electrophoresis (0 ' Farrell

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1975) and transferred onto PVDF membrane. Western blotting analysis was performed by

applying the primary antibody containing anti-nicotianamine synthase antibody prepared in

example 1 example 9 and the secondary antibody containing horse radish binding anti-mouse

IgG (H + L) goat antibody (Wako Pure Chemicals Co.) on the membrane and coloring with

diaminobenzidin.--

On page 28 of the English translation, please substitute the sequence listing with the

substitute sheets (32 pages) attached hereto and renumber the pages with the claims and the

abstract accordingly.